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# Smells good enough to eat: Dimethyl sulfide (DMS) enhances copepod ingestion of microplastics



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#### ABSTRACT

Marine copepods have been shown to readily ingest microplastics - a crucial first step in the transfer of plastics into the marine food chain. Copepods have also been shown to elicit a foraging behavioural response to the presence of olfactory stimuli, such as dimethyl sulfide (DMS) – a volatile compound produced by their algal prey. Here, we show that the temperate Calanoid copepod *Calanus helgolandicus* displays enhanced grazing rates of between 0.7 and 3-fold (72%–292%) on microplastics that have been infused in a DMS solution, compared to DMS-free controls. Environmental exposure of microplastics may result in the development of an olfactory signature that includes algal-derived compounds such as DMS. Our study provides evidence that copepods, which are known to use chemosensory mechanisms to identify and locate dense sources of palatable prey, may be at an increased risk of plastic ingestion if it mimics the scent of their prey.

#### 1. Introduction

Microplastics (microscopic plastic, 0.1 µm-5 mm) are of major environmental concern because their microscopic size makes them bioavailable to a wide range of marine organisms across trophic levels (Cole et al., 2013; Allen et al., 2017). Many factors can influence the bioavailability of microplastics within the marine ecosystem, such as density, size and abundance (Wright et al., 2013). However, the act of ingesting microplastic in the natural environment depends upon the likelihood of a particular marine species encountering and interacting with the microplastic particles and the susceptibility of that species (Engler, 2012; Setälä et al., 2018). It has been hypothesised that many marine species mistakenly identify plastic debris as a food source due to the similar characteristics of the plastic and prey. For example, fulmars may mistake floating plastics debris for cuttlebones (Cadée, 2002), whilst leatherback turtles may misidentify soft plastic debris as gelatinous prey organisms (Schuyler et al., 2014). Recent evidence also suggests complex chemosensory cues, involving volatile compounds such as dimethyl sulfide (DMS), may be responsible for mediating foraging behaviour and consumption of marine plastic debris (Savoca et al., 2016; Allen et al., 2017; Savoca et al., 2017).

DMS concentrations in the surface ocean typically range from 1 to 7 nM, and in the north Atlantic Ocean DMS concentrations peak during June–July, corresponding with the annual coccolithophore and

dinoflagellate bloom (Lana et al., 2011). These elevated seawater concentrations result in enhanced atmospheric DMS concentrations, which may create an olfactory map upon the featureless ocean surface, providing chemosensory species with an efficient means of identifying the location of dense, palatable prey (Nevitt et al., 1995). These dense patches of primary productivity are often associated with oceanic features such as seamounts, shelf breaks, coastal zones and upwelling zones where nutrients are plentiful (Nevitt, 2008). It has recently been established that a variety of species demonstrate foraging behaviour in the presence of DMS including whale sharks, Rhincodon typus (Dove, 2015), loggerhead sea turtles, Caretta caretta (Endres and Lohmann, 2012), African penguins, Spheniscus demersus (Wright et al., 2011), harbour seals, Phoca vitulina vitulina (Kowalewsky et al., 2006), hard coral, Astrangia poculatia (Allen et al., 2017), northern anchovy, Engraulis mordax (Savoca et al., 2017) and the marine microbial community (Seymour et al., 2010). Evidence suggests that species that predominately predate upon planktonic prey use DMS as an infochemical to locate areas of dense primary productivity. This includes planktonic secondary producers such as zooplankton, in particular copepods, which are dominant members of the zooplankton community. Copepods provide a fundamental link in the food chain, consuming energy from primary producers, transferring this energy to higher trophic levels and playing an important role in recycling and remineralising organic materials (Blaxter et al., 1998; Harris et al., 2000).

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Furthermore, copepods employ both chemo- and mechano-reception feeding mechanisms to identify the location of palatable prey and to increase grazing success while also conserving energy (Blaxter et al., 1998; Steinke et al., 2006). Considering the important role that copepods play in the marine ecosystem, identifying those species and ecosystems at greatest risk from microplastic contamination requires a better understanding of the mechanisms which influence microplastic ingestion by copepods in the natural environment. Previous laboratory research has established that virgin microplastics are readily ingested by a range of marine biota across trophic levels including mussels (Browne et al., 2008), decapod crustaceans (Watts et al., 2014), corals (Allen et al., 2017), fish (Foekema et al., 2013) and copepods (Cole et al., 2013). Copepods exposed to varying treatments of algal prev and virgin microplastics demonstrated impeded algal ingestion in just 24 h. This resulted in a reduction in the number of algal cells and total carbon biomass ingested (Cole et al., 2013); Cole et al. (2015) found copepods exposed to microplastics displayed a significant reduction in the size of eggs produced and in hatching success and ultimately survival. This could potentially lead to energy deficiencies and reduced growth and therefore limit secondary production. These effects could impose detrimental impacts upon species of a higher trophic level that depend on copepods as a food source. It has also been recently discovered that microplastic ingestion in copepods significantly reduced the density of their faecal pellets (Cole et al., 2016), an important source of nutrients, carbon and energy to deeper waters.

In this study we aim to improve our understanding of the ingestion of microplastic by copepods by testing the hypothesis that Calanoid copepods display enhanced feeding rates on exposure to DMS-infused microplastics, compared to DMS-free controls. Our study design involved 6 hour feeding experiments using  $10\times30\,\mu m$  nylon microfibres at a concentration of 100 microfibres mL $^{-1}$ . We used natural specimens of a marine copepod *Calanus helgolandicus*, a species common to the temperate northeast Atlantic. We discuss our findings in relation to the role of volatile infochemicals in influencing the ingestion of microplastics by marine organisms.

# 2. Materials and methods

### 2.1. Zooplankton sampling and husbandry

Zooplankton samples were collected from the Western Channel Observatory stations L4 (50°15′N, 4°13′W) and E1 (50°03′N, 4°22′W) using WP2 (57 cm diameter, 200  $\mu m$  mesh) plankton nets from June to August 2017. Samples were kept in an insulated container and transferred to Plymouth Marine Laboratory within three hours of collection. Zooplankton samples were examined under a dissecting microscope and adult female *Calanus helgolandicus* were identified through assessment of their life stage, size, shape and presence of a distinct genital pore. Individuals were carefully picked out and transferred to 5 litre beakers containing filtered seawater. All samples were processed and experiments conducted within a controlled temperature (CT) laboratory matched to the sea surface temperature of 15  $\pm$  1 °C.

#### 2.2. Preparation of microplastics

Fresh cut virgin nylon fibres ( $10 \times 30 \,\mu\text{m}$ ) were chosen as the most environmentally representative microplastic that are available (Cole et al., 2016). Experimental flasks ( $500 \, \text{mL}$ ) were filled to the brim with 615 mL of filtered seawater and spiked with ~80 fibres mL $^{-1}$  of virgin (control) or DMS-infused nylon microfibers. DMS-infused nylon microfibers were prepared by infusion in a 5 nM DMS solution, prepared by serial dilution of pure DMS (Sigma Aldrich Company Ltd.) in MilliQ water, in 5 mL gas-tight vials for 7 days in a refrigerator. Non-infused virgin microfibers were prepared identically, with the omission of the addition of DMS. All vials were kept at 5 °C for 7 days before use in grazing experiments. When added to the experimental flasks, the 5 mL

addition of the DMS-infused nylon microfibres in 5 nM DMS solution resulted in an increase in ambient DMS concentrations of approximately 0.7 nM.

#### 2.3. Grazing experiments

The copepod grazing experiments consisted of a DMS-free microplastic control group and a DMS-infused microplastic treatment group with up to ten replicates for each treatment. Grazing experiment one (GE1) consisted of six replicates per treatment, using copepods that had been acclimated for 14 days, while grazing experiment two (GE2) consisted of ten replicates per treatment using copepods that had been acclimated for two days. The unicellular alga, Dunaliella tertiolecta, taken from culture maintained on F/2 media at 15 °C, was provided as a source of prey for the copepods on alternative days during the acclimation period. Copepods for both GE1 and GE2 were removed from culture for a starvation period in filtered seawater for approximately 18 h before experimental set-up. The target concentrations of microplastics and DMS were the same for each experiment. Grazing experiments were carried out in air tight, 500 mL Pyrex bottles (actual total volume 615 mL) filled to the brim with 0.2 µm filtered seawater (FSW). Five healthy adult female C. helgolandicus were transferred to each experimental bottle, followed by the addition of microplastics, with or without DMS. Copepods were not added to T<sub>0</sub> incubation bottles. The experimental bottles were secured to a plankton wheel, rotated at < 5 rpm and left for 6 h in the dark in a laboratory maintained at ambient sea temperature (15-16 °C). After 6 h the experiment was stopped and the copepods were removed from each experimental bottle by gently passing the bottle contents through a 150 µm mesh into a beaker. The experimental water was returned to the experimental bottles which were then stored at 4°C prior to microplastic enumeration using FlowCam (Fluid Imaging Technologies Ltd.). The mesh containing the copepods was placed in shallow Petri dish containing filtered seawater; each copepod was checked using a dissecting microscope and any mortality was recorded.

A FlowCam (VS-4 series) fitted with a 100  $\mu m \times 2$  mm flow cell and 10 x objective was used to determine the concentration of microplastics in each experimental bottle at  $T_0$  and  $T_6$ . Analyses were carried out using autoimage mode at a rate of 1.75 mL min  $^{-1}$  and an image capture rate of 20 frames per second. Following the analysis, images of microplastics were characterised using VisualSpreadsheet software (v 4.0). Copepod grazing rates were estimated by comparing changes in the abundance of microplastics over the experimental period with and without the addition of DMS. *C. helgolandicus* ingestion rates (fibres copepod  $^{-1}$  day  $^{-1}$ ) were calculated using an adapted version of the Frost (1972) equation which accounted for the absence of prey growth during the incubations.

The grazing coefficient (g) was calculated from:

$$0 - \log \frac{T6}{T0} x \frac{1}{T} \tag{1}$$

where  $T_0$  is the concentration of fibres  $\mathrm{mL}^{-1}$  at the start of the experiment and  $T_6$  is the concentration of fibres  $\mathrm{mL}^{-1}$  post-grazing experiment and T is time in hours. The clearance rates F (mL copepod  $^{-1}$  h  $^{-1}$ ) were then calculated from:

$$F = \frac{V \times g}{n} \tag{2}$$

where V is the volume of the incubation bottle (mL), g is the grazing coefficient calculated above and n is the number of copepods in the treatment bottle. The ingestion rate I is then calculated as (T<sub>0</sub> prey/microfibre concentration):

$$I = F \times T_0 \tag{3}$$

#### 2.4. Statistical analysis

All data was analysed using Microsoft Excel (2016). A two-way student's t-test assuming unequal variance was used to compare experimental data from grazing experiments. The significance level was defined at  $\alpha = 0.05$ .

#### 2.5. Experimental microplastic DMS infusion

A separate experiment was undertaken in order to test the uptake of DMS onto the microfibres. Nylon microfibres were infused in a DMS solution in gas-tight vials and the concentration of DMS in the water phase was monitored over the course of 10 days, and compared to vials that did not contain microfibres. Any change in water phase DMS concentration in the vials containing microfibres could indicate adsorption/uptake onto the nylon. Specifically, five 8 mL serum vials were filled with 5 mL MilliQ water and 3 mL of nylon fibres ( $10 \times 30 \,\mu m$ ) primary stock (estimated concentration  $3.36 \times 10^6 \, \text{fibres mL}^{-1}$ ) thus, generating a final concentration in the vials of  $\sim 1.00 \times 10^7$  fibres mL<sup>-1</sup>. Each vial was crimp sealed and received an addition of 5 µL DMS solution to achieve a concentration of 1.2–1.3 nM of DMS per vial. This was repeated for the microplastic-free controls, with an addition of 3 mL of MilliQ water replacing the addition of nylon fibres. Vials were analysed via gas chromatography (Varian 3800) with pulsed flame photometric detection (GC-PFPD) immediately after the addition of DMS (see below). All remaining samples were stored at 5 °C until analysis at five time points (approximately every 48 h) over a period of 240 h.

#### 2.6. DMS analysis

DMS analysis for the infusion experiment was carried out using a Varian 3800 gas chromatograph with pulsed flame photometric detector (GC-PFPD) using published methods (Archer et al., 2013, Hopkins and Archer, 2014). A 5 mL sample was withdrawn from the vial through the septum using a needle connected to a glass syringe with a PTFE Luer valve. An additional needle was inserted to allow for air exchange. The sample was then filtered directly into a 2 mL syringe through a 25 mm 0.6  $\mu$ m GF/F (Whatmann) filter paper held within an inline Swinnex filter unit, in order to remove the microplastics while also preventing the loss of DMS via exposure to air. Samples were analysed using cryogenic purge and trap, purging with Helium at 60 mL min  $^{-1}$  for 5 min and trapping in a PTFE loop submerged in liquid nitrogen, following by desorption and quantification via GC-PFPD (Archer et al., 2013).

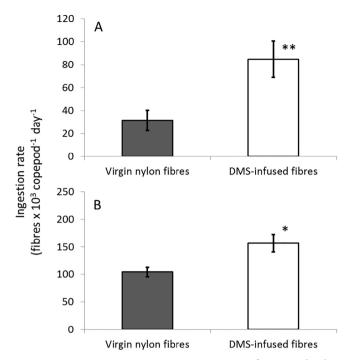
#### 3. Results

#### 3.1. Grazing experiments

The copepod *Calanus helgolandicus* demonstrated a significantly higher ingestion rate on DMS-infused nylon microfibres compared to DMS-free microfibres over the course of both grazing experiments (Fig. 1 and Table 1). For grazing experiment 1 (GE1), DMS-infused fibres were ingested at a mean rate ( $\pm$ 1 SD) of 84.62 × 10<sup>3</sup> ( $\pm$ 15.75 × 10<sup>3</sup>) fibres copepod<sup>-1</sup> day<sup>-1</sup>, compared to a mean ( $\pm$ 1 SD) of 31.38 × 10<sup>3</sup> ( $\pm$ 8.88 × 10<sup>3</sup>) fibres copepod<sup>-1</sup> day<sup>-1</sup> for the DMS-free control (Fig. 1A, *t*-test, *P* < 0.01). For grazing experiment 2 (GE2), ingestion rates on DMS-infused nylon microfibres of 15.68 × 10<sup>4</sup> (18.37 × 10<sup>3</sup>) fibres copepod<sup>-1</sup> day<sup>-1</sup> were significantly higher rates from than the DMS-free control (10.41 × 10<sup>4</sup> (15.38 × 10<sup>3</sup>)) fibres copepod<sup>-1</sup> day<sup>-1</sup> (Fig. 1B, *t*-test, *P* < 0.05).

# 3.2. Microplastic DMS infusion experiment

In order to test the uptake/adsorption of DMS by nylon microfibres,



**Fig. 1.** Calanus helgolandicus ingestion rates (fibres  $\times$  10<sup>3</sup> copepod<sup>-1</sup> d<sup>-1</sup>) of virgin and DMS-infused microfibres from two grazing experiments (A GE1, B GE2). Error bars show  $\pm$  1 SD from the mean. Asterisks denote levels of significance of difference in grazing rate between the two treatments,  $^*=P \le 0.05$ ,  $^{**}=P \le 0.01$ .

microfibres were infused in a 1.2-1.3 nM DMS solution for 240 h (10 days), and compared to DMS-only controls. We hypothesised that any measured loss of DMS from the solutions could be attributed to uptake/adsorption by the nylon microfibres, when compared to the controls. In reality, we observed loss of DMS from both sets of vials, although the rate of loss was enhanced in the presence of nylon microfibres (Fig. 2). In the presence of microfibres, a maximum reduction in DMS of 0.4 nM was observed after 144 h, compared to 0.2 nM in the DMS-only controls (Fig. 2). The greatest differences in loss rate between the controls and DMS + microfibres samples were seen over the first 144 h, with a rate of 0.03 nM/d for DMS only, and 0.07 nM/d for DMS + microfibres. Overall, there was some evidence of enhanced loss of water phase DMS in the presence of nylon microfibres, potentially due to uptake/adsorption onto the nylon. After 144 h, the difference between the two treatments decreased. The cause of this is unclear, but it may be indicative of the microfibres approaching equilibrium with the DMS solution. Over the 240 h infusion, loss rates of 0.03 nM/d from the DMS-only controls were observed. This baseline loss of DMS could represent either loss of DMS from the vials via gaseous diffusion, and/or microbial DMS uptake as it is likely the vials and MilliQ water used were not completely bacteria-free.

Whilst this small scale, preliminary experiment provides intriguing information regarding the uptake of DMS onto the microfibres, we recognise the limitations associated with this data. A major limitation was the availability of nylon microfibres – a very high concentration of microfibres ( $1.0 \times 10^7$  fibres mL $^{-1}$ ) was required before we saw any detectable change in water phase DMS concentrations, and this meant the experimental design was limited to single samples per time point for the DMS + microfibres samples, generating some uncertainty in the measurements. In the future, greater availability of microfibres would allow a higher level of replication, providing a greater level of certainty associated with such data.

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Table 1 Summary of the mean ( $\pm 1$  SD) clearance rates (mL copepod<sup>-1</sup> d<sup>-1</sup>) and mean ( $\pm 1$  SD) ingestion rates ( $\times 10^3$  fibres copepod<sup>-1</sup> d<sup>-1</sup>), including the level of significance of differences in ingestion rates between treatments, from the two grazing experiments in this study.

	Experiment 1		Experiment 2	
	Virgin nylon fibres	DMS-infused nylon fibres	Virgin nylon fibres	DMS-infused nylon fibres
Average clearance rate (mL copepod <sup><math>^{-1}</math></sup> d <sup><math>^{-1}</math></sup> ) Average ingestion rate ( $\times 10^3$ fibres copepod <sup><math>^{-1}</math></sup> d <sup><math>^{-1}</math></sup> ) Significance test (ingestion rate)	$149.10 \pm 18.8$ $31.38 \pm 8.88$ $t = 2.76, df = 8, P = 0.0$	402.01 ± 33.4 84.62 ± 15.75	$494.6 \pm 73.0$ $104.1 \pm 15.38$ t = 1.78, $df = 14$ , $P = 0$	745.1 ± 27.6 156.8 ± 18.37

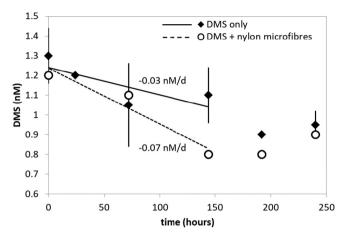


Fig. 2. Water phase DMS concentrations (nM) in gas-tight vials in the presence (open circles) and absence (closed diamonds with error bars which show  $\pm$  1 SD) of nylon microfibres over the course of 244 h. Regression lines show loss rates of DMS over the first 144 h in the presence of DMS (dashed line) and for DMS-free controls (solid line).

# 4. Discussion

The results from both grazing rate experiments in this study provide first evidence that the temperate copepod *Calanus helgolandicus* is stimulated to graze upon nylon microfibres that have been infused in DMS solution. This is shown by the significant 0.7–3 fold increase in ingestion rates of copepods exposed to DMS-infused nylon microfibres (treatment group) compared to copepods exposed to virgin nylon microfibres (control group), in two separate experiments. These results imply that the increased ingestion rates seen for copepods exposed to DMS-infused nylon microfibres compared to virgin nylon microfibres was most likely a consequence of the DMS being absorbed from the DMS solution to the surface of the nylon microfibres prior to the grazing experiments, and thence acting as a chemical cue to attract the copepod to consume more 'prey' or in the case of this study, microfibres 'smelling' of prey.

Recent research has demonstrated that DMS plumes stimulated a grazing behavioural response of C. helgolandicus at concentrations of 1.8 nM to 13.1 nM DMS, obtained from low and high DMSP producing strains of E. huxleyi, respectively (Breckels et al., 2013). Therefore if, as demonstrated in this study, microplastics possess the ability to adsorb and re-emit volatile infochemicals from their surrounding environment, this could render them more palatable to copepods and could result in them entering the food chain more readily. Whilst recognising the limitations of our infusion experiment, our results suggest that copepods may be able to detect very low concentrations of DMS. Our data implies that the uptake per microfibre was in the femtomolar range  $(\times 10^{-15})$ , which is two orders of magnitude lower than the nanomolar levels reported by Breckels et al. (2013). The handling and filtration of the seawater used for the grazing experiments results in significant outgassing of DMS, such that very low or undetectable levels remain following this procedure. We performed some separate tests to confirm this. Concentrations of DMS in seawater were determined in triplicate samples before and after filtering, and we saw a reduction in DMS from  $0.2 \pm 0.09\,\mathrm{nM}$  to  $0.04 \pm 0.08\,\mathrm{nM}$ , with undetectable levels of DMS in two of the three post-filtration samples. Thus, we can assume that the relative difference between ambient DMS concentrations in the surrounding seawater and the 'hotspots' created by the DMS-infused microfibres was great enough to induce the copepods to ingest the fibres.

#### 4.1. Microplastic ingestion by C. helgolandicus

The copepod C. helgolandicus has been the subject of multiple grazing studies and is known to feed on a diverse array of prey, including phytoplankton and protozoan microzooplankton (Irigoien et al., 2000; Fileman et al., 2007; Djeghri et al., 2018). It has also been established that copepods can discriminate between preferred types of prey when presented with a choice based on size (Frost, 1972), species (Paffenhöfer, 1971) and nutritional quality (Cowles et al., 1988), to such an extent that copepod grazing activities can influence the composition of the microbial food web (Fileman et al., 2010). Despite the ability of copepods to demonstrate a prey preference based on many variables, and the capacity to distinguish between types of prey, C. helgolandicus are known to feed less selectively when they have reached food satiation or are starved (Fileman et al., 2007). Therefore, the fact that in this study copepods were still shown to ingest virgin nylon microfibres, may have been a result of the starvation period prior to exposure and/or because the fibres are of a similar size to their natural prey source (Hassett and Landry, 1988).

Recent literature has demonstrated that DMS stimulates foraging behaviour in predators and secondary consumers (Savoca et al., 2016; Savoca et al., 2017; Breckels et al., 2013) and the results here confirm that microfibres that have been infused in DMS solutions at environmentally-relevant concentrations are a grazing stimulus for Calanoid copepods. Future work could consider whether different shapes, sizes and colours of microplastics also influence copepod ingestion rates and if the presence of a DMS cue overrides this selection process. The importance of the presence of a biofilm should also be considered. particularly in the context of the production of DMS by bacteria and algae inhabiting the biofilm. Nevertheless, given the varying degree of prey selectivity that has been observed throughout different species of copepods, as well as observations of individual variability (Vroom et al., 2017), the use of DMS as a single variable, rather than including the complexity of a developed biofilm removes variation of independent differential attractiveness. This helps to isolate the response to DMS alone, implicating it as an important infochemical in the feeding ecology of copepods.

#### 4.2. Infusion of nylon microfibres with DMS

The results of this study suggest that nylon microfibres absorb a detectable amount of DMS after 216 h. Savoca et al. (2016) found that three different types of plastic exposed to the marine environment for three weeks emitted a DMS signature within the range of 0.6–28  $\mu g$  DMS per gram of plastic. However, it was unclear whether the DMS was emitted from the biofilm adhered to the plastic or from the plastic material itself. A recent study showed that a variety of copepod species more readily ingested microplastics that had been aged in seawater for

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3 weeks compared to virgin microplastics, and this was indirectly attributed to the formation of the biofilm which may have contained algal prey, as well as the secretion of chemical exudates that aided chemoattraction (Vroom et al., 2017). However, no measurements were made of chemical exudates, and further investigation of the influence that biofilm formation may have upon copepod ingestion rates is warranted. Our study implies that biofilm formation is not essential to increasing the palatability of microplastics to marine grazers such as copepods, and the release of chemical cues may play a key role.

There is currently a significant knowledge gap regarding the ability of marine plastic debris to gain an infochemical signature via adsorption and through the establishment of a biofilm. There is no reported research on the infochemical signature of marine plastic debris and the potential influence this has on increasing the likelihood of ingestion by marine wildlife. Engler (2012) reported there was no statistically significant difference between the adsorption properties of weathered and virgin plastic. However, it was found that weathered polyethylene tended to adsorb an increased amount of pollutants compared to virgin polyethylene. This highlights the need for future research to investigate the adsorption capabilities of different types of plastic in relation to the different type of infochemicals, as well as different types of plastics that have been aged in the natural environment.

# 4.3. The influence of DMS on copepod grazing

Our findings provide evidence that nylon microfibres may have the ability to acquire volatile infochemicals from their surrounding environment. This could create a hotspot of DMS around the external surface of the microfibres similar to the diffusion limited concentration gradient found in the pycosphere of phytoplankton cells (Breckels et al., 2010). Thus, DMS-emitting microfibres may replicate the mechanisms via which copepods identify palatable prey in the natural environment, resulting in elevated ingestion rates on DMS-infused microfibres.

DMS is the volatile by-product of the breakdown of the algal osmolyte dimethyl sulfoniopropionate (DMSP). Intracellular DMSP concentrations can vary considerably between both different species and different strains of the same species of phytoplankton (Franklin et al., 2010; Archer et al., 2011). Healthy cells continually exude DMSP thus enhancing the concentration within the pycosphere above background levels, with this rate of exudation increasing under stress, grazing or viral lysis (Breckels et al., 2010). Once outside the cell, DMSP is rapidly broken down to DMS, either via DMSP lyase enzymes associated with the algal cell or in the surrounding water (Steinke et al., 1998), or via bacterial catabolism (Moran et al., 2012). This creates a hotspot of DMSP and DMS close to the cell, and serves as a potential chemoattractant for copepod predators. C. helgolandicus has demonstrated a foraging behavioural response to DMS in a concentration range from 1.8-13.1 nM (Breckels et al., 2013), although our results imply that the threshold of detection by calanoid copepods may be much lower, possibly within the femtomolar range. There is also likely to be a high degree of variability between the responses of individual copepods (Breckels et al., 2013; Vroom et al., 2017), which could explain some of the variability found in the results of this study. Further research is required to identify the detection threshold of chemosensory marine organisms.

#### 5. Conclusions

This study tested the hypothesis that the calanoid copepod *C. helgolandicus* would be stimulated to graze upon nylon microfibres that had been infused in an artificial DMS solution with an environmentally-relevant concentration. By measuring the differences between the ingestion rates of copepods exposed to virgin nylon microfibres against those exposed to DMS-infused nylon microfibres, our results provide first evidence that copepods are stimulated to graze upon microfibres that have been exposed to DMS. Research has only recently begun to

uncover the chemosensory mechanisms of marine organisms, including apex predators and primary consumers. Our results suggest that chemosensory species utilising DMS as an infochemical may be at heightened risk of consuming plastic debris. However, this area of research requires further investigation in order to increase our understanding of the interspecies response to differing infochemicals and the detection thresholds of chemosensory marine organisms.

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